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Karyological investigations of *Caryophyllaeus laticeps* (Pallas, 1781) (Cestoda: Caryophyllidea)

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Abstract. Mitotic and meiotic chromosomes of *Caryophyllaeus laticeps* (Pallas) from Rybinsk reservoir were studied using air-drying techniques. It was established that the diploid set contains 20 metacentric chromosomes. The chromosomes are large – their mean absolute length ranges from 3.00 to 12.90 μm. The last pair of small homologues comprises only 3.4 % of the total length of the complement. One specimen of *C. laticeps* with 2n = 21 (trisomic after the last pair of chromosomes) and one triploid specimen with 3n = 31 is described. Data on the comparative karyology of caryophyllids are discussed.

Many questions on the systematics and phylogeny of caryophyllids are controversial (Bazitov 1981, Mackiewicz 1982), and undoubtedly some of them can be solved with the help of cytogenetic studies. Relatively large chromosomes and frequently occurring mitotic plates in preparations are characteristic of caryophyllids, unlike most cestodes, which facilitates karyological analysis. However, during the last decade no papers have been published on the karyology of this group of helminths. There is some older information in the literature on the chromosome sets of six species: *Archigetes appendiculatus*, 2n = 18 (Motomura 1929), *Atractolytocestus huronensis*, 3n = 24 (Jones and Mackiewicz 1969), *Hunterella nodulosa*, 2n = 14 (Mackiewicz and Jones 1969), *Glaridacris laruei*, 2n = 16 (Grey and Mackiewicz 1974), *G. catostomi*, 2n = 20, 3n = 30 (Grey and Mackiewicz 1980), *Lytocestus indicus*, 2n = 16 (Vijayaraghavan and Subramanyam 1977). Mackiewicz (1981) gave a list of 20 species of caryophyllids with chromosome numbers. Besides the reports mentioned above, there are the unpublished results of a cytological investigation of 14 additional species of caryophyllidean cestodes, carried out by Grey (unpublished Ph. D. thesis, State University of New York at Albany, 1979, Dissertation Abstracts International B40, pp. 590–591). But no other information on the chromosome complements of these species has been published subsequently.

We have studied a chromosome set of another species, *Caryophyllaeus laticeps* Pallas, 1781, a widespread parasite of cyprinid fishes, in order to extend the scanty karyological knowledge of Caryophyllidea.

MATERIALS AND METHODS

Chromosomes were studied on preparations of 23 specimens of *C. laticeps* obtained from the intestines of bream (*Abramis brama* L.) collected from the Rybinsk reservoir (Yaroslavl region, Russia) in May 1989.

Living helminths were treated with 0.01 % colchicine in physiological solution (0.65 % NaCl) for 2–3 h at room temperature. They were then fixed in a freshly prepared fixative of 96 % ethylalcohol and glacial acetid acid (3 : 1). The fixed material was stored at 4 °C for a month. Chromosomal preparations were made by the air-dried method from cellular suspension of the whole worm (Petkevičiūtė and Ieshko 1991) not earlier than after 24 h fixation. Prior to staining, the preparations were placed into 1N HCl for 10–15 min and then rinsed three times in distilled water. Chromosomes were stained with 4 % Giemsa in phosphate buffer (pH 6.8) for 30–40 min.

Karyometric analysis was carried out on karyotypes composed from photographs of 10 metaphase plates containing well spread chromosomes. The following parameters were defined: the absolute length of chromosomes in μm, the relative length (the relation of chromosome length to total length of the haploid in percentage terms), and the centromeric index (the relation of the shorter arm to the entire chromosome length expressed in percentage). Means are given with standard deviations.

The chromosome classification employed is that of Levan et al. (1964).

RESULTS

Karyological analysis demonstrated that the modal number of chromosomes in cells of 21 specimens of *C. laticeps* (out of 23 being studied) is 20 at the stage of mitotic metaphase. Haploid sets with n = 10 at various stages of meiotic division confirmed the diploid number. A total of 69 mitotic cells (54 of the contained

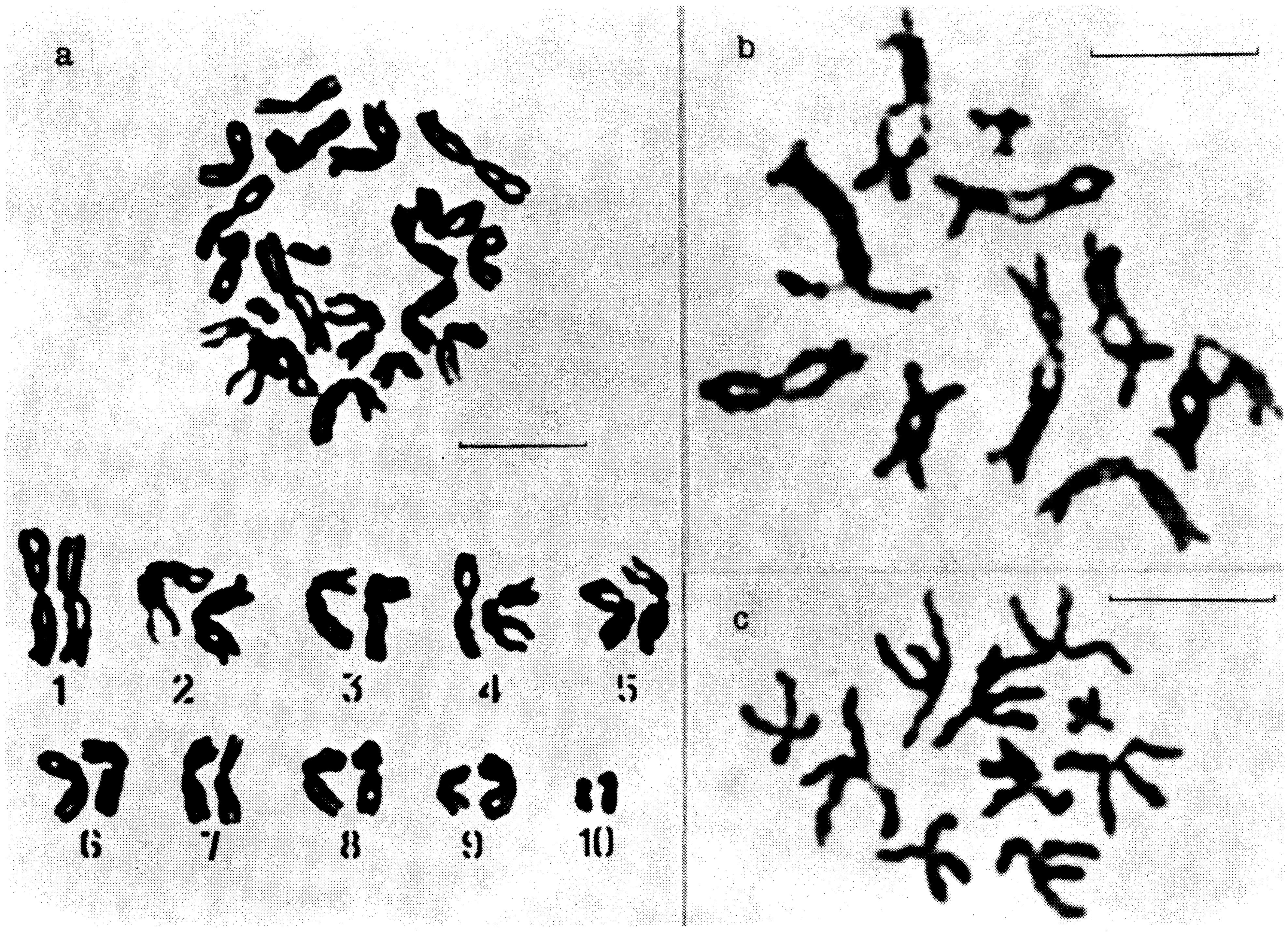

Fig. 1. Mitotic and meiotic chromosomes of *C. laticeps*. a – mitotic metaphase plate and karyotype, 2n = 20; b – diakinesis of meiosis, showing 10 bivalents with chiasmata; c – metaphase II of meiosis, n = 10. Scales are 10 μm.

20 chromosomes, 10 were aneuploid and 5 polyploid) and 140 meiotic cells (125 of them contained 10 structural units, 11 were aneuploid and 4 polyploid) from these specimens were studied.

Table 1. Measurements (means ± SD) and classification of chromosomes of *Caryophyllaeus laticeps*

Chromosome number	Absolute length (μm)	Relative length (%)	Centromeric index	Classification
1	12.91 ± 2.44	14.66 ± 0.67	44.44 ± 2.39	m
2	11.41 ± 2.19	12.94 ± 0.60	44.96 ± 3.00	m
3	10.23 ± 1.91	11.61 ± 0.34	44.12 ± 2.19	m
4	9.78 ± 1.82	11.11 ± 0.48	43.95 ± 1.41	m
5	9.24 ± 1.38	10.57 ± 0.43	45.36 ± 2.22	m
6	8.73 ± 1.45	9.95 ± 0.38	45.88 ± 1.20	m
7	8.05 ± 1.22	9.21 ± 0.50	44.91 ± 2.06	m
8	7.76 ± 1.42	8.81 ± 0.41	46.39 ± 1.47	m
9	6.75 ± 1.09	7.71 ± 0.49	43.88 ± 2.75	m
10	2.97 ± 0.32	3.43 ± 0.38	42.50 ± 3.14	m

m – metacentric chromosomes.

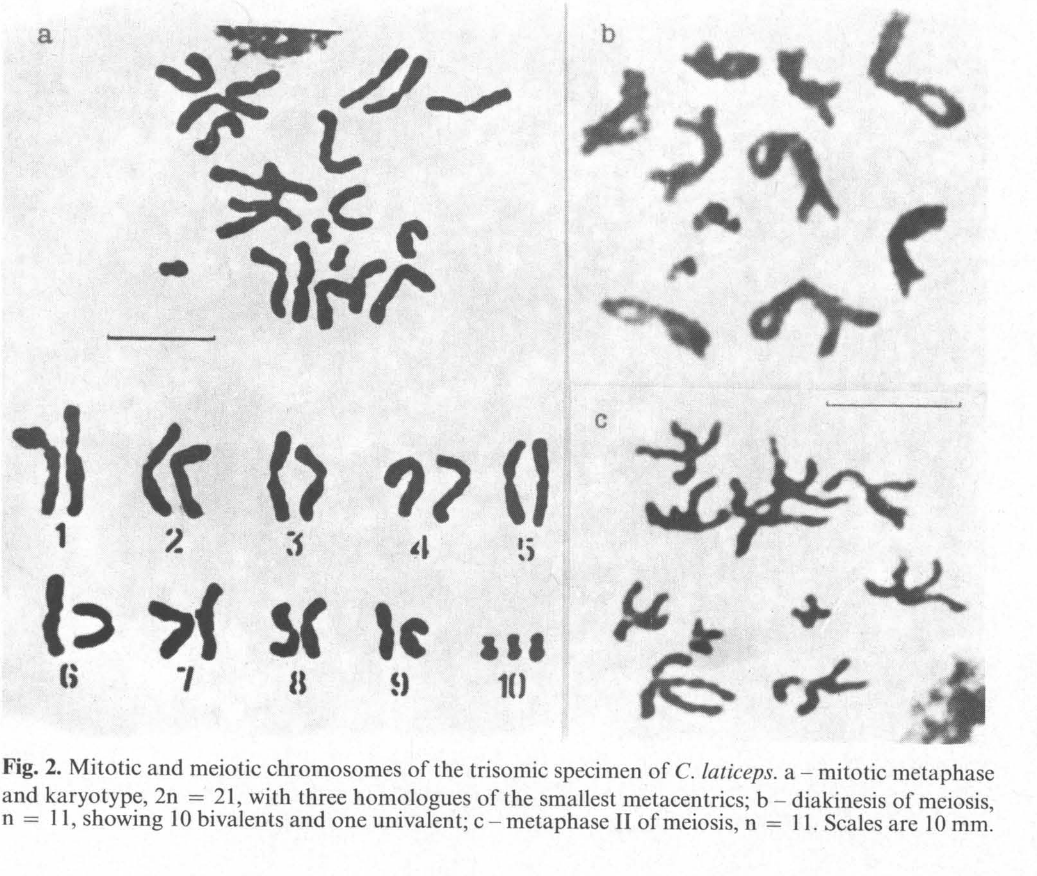

Fig. 2. Mitotic and meiotic chromosomes of the trisomic specimen of *C. laticeps*. a – mitotic metaphase and karyotype, 2n = 21, with three homologues of the smallest metacentrics; b – diakinesis of meiosis, n = 11, showing 10 bivalents and one univalent; c – metaphase II of meiosis, n = 11. Scales are 10 mm.

The karyotype of *C. laticeps* consists of large chromosomes: their mean length extends from 3.00 to 12.90 μm (Table 1). The mean total chromosome length of the haploid complement is 87.83 μm. All chromosomes are metacentric (Fig. 1). The homologues of many pairs could not be distinguished clearly; therefore, the chromosomes pairs 3 and 4, 4 and 5, 5 and 6, 6 and 7, and 7 and 8 have no statistically significant differences in their sizes and centromeric indices. On the contrary, the chromosomes of the last, 10th pair, stand out sharply among the other elements of the genome because of their short length.

The preparations contained many cells at the stage of diakinesis and metaphase II (Fig. 1b, c). The chiasma frequency, calculated from counts made on 20 cells, showed that the mean number of chiasmata/cell is 32.8. The chiasma frequency on a bivalent varied from 1–2 in the smallest metacentrics to 5 in large chromosomes.

There were 21 chromosomes in the diploid set of one of the helminths examined. Observations were made on 16 mitotic cells; 14 of them contained 21 chromosomes. The analysis showed that this specimen was a trisomic after the last pair of small

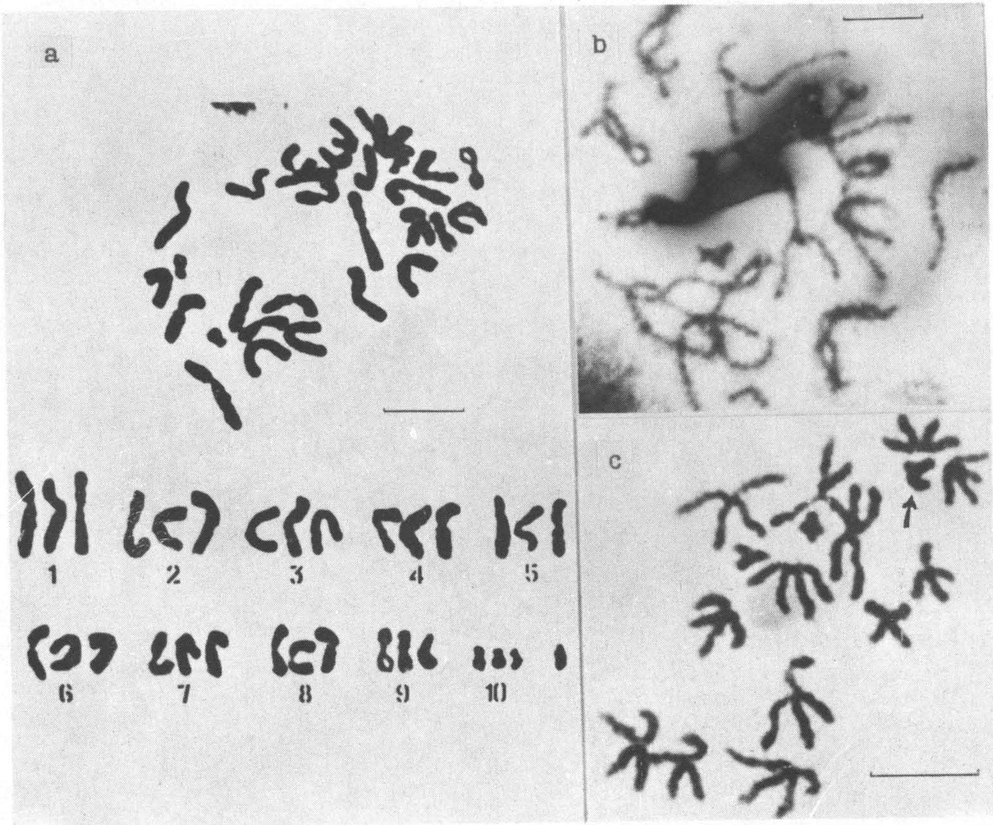

Fig. 3. Mitotic and meiotic chromosomes of triploid specimen of *C. laticeps*. a – mitotic metaphase and karyotype, 3n = 31; b – diakinesis of meiosis showing bivalents and univalents; c – metaphase II of meiosis, n = 16; arrow indicates additional subtelocentric chromosome. Scales are 10 μm.

metacentric chromosomes (Fig. 2a). At the stages of diakinesis and metaphase II in most cells (20 of 26 studied) there were found 11 structural units (Fig. 2b, c).

Triploid was one of the helminths studied. In addition to three haploid sets of chromosomes, another additional element was found in most of the cells investigated (3n = 31). A total of 19 mitotic plates was examined; 14 of them contained 31 chromosomes, the other 5 cells contained 28–30 chromosomes. There was no homologue for the additional 31st element in the chromosome complement. This single element has a subtelocentric type of structure and its length somewhat exceeded that of the smallest chromosomes of the complement. (Fig. 3a). At the stages of diplotene and diakinesis in the cells along with bivalents nonconjugating univalents were also evident (Fig. 3b). At the stage of metaphase II in most cells, 16 elements were observed, and one subtelocentric element was seen among other metacentric chromosomes (Fig. 3c). Some meiotic cells of this specimen contained a hexaploid set of chromosomes (n = 30).

DISCUSSION

Karyology has been succesfully applied to the systematics of many groups of organisms as a differentiating tool for the characterization of species and intraspecific groups, and as an integrating feature in analysing the interrelationships among supraspecific taxonomic groups. Unfortunately, our knowledge of the structure of chromosome sets of caryophyllids is still limited and existing data cannot provide enough basis for generalizations on phylogenetic relationships of species both within Caryophyllidea or within other groups of cestodes.

According to available data, one karyological pecularity of caryophyllids is large chromosomes, a fact that is also confirmed by investigation of the chromosomes of *C. laticeps*. The diploid number of chromosomes of the species described in the literature varies from 14 to 20. Mackiewicz (1981, 1982), on the basis of unpublished data, reports that diploid chromosome sets of caryophyllids contain from 6 to 20 elements (the amplitude of variability of the chromosome number of other cestodes is from 8 to 28). The number of chromosomes reported for *C. laticeps*, 3n = 30 (Grey 1979, cit. in Mackiewicz 1981), pointed to the existance of a triploid form of that species, but no karyological details of the karyotype structure were supplied.

The comparison of the structure of karyotypes showed that there is a very close caryological affinity between *C. laticeps* and *Glaridacris catostomi.* The karyotype of *G. catostomi* consists of 20 biarmed (8 pairs of metacentric and 2 pairs of submetacentric) chromosomes, the length of which ranges from 3 to 8 μm (Grey and Mackiewicz 1980). The chromosomes of *C. laticeps* are exclusively metacentric and somewhat larger – up to 12.9 μm, but it should be noted that the differences in the size of chromosomes could partially be caused by different methods of preparation. The karyotypes of these two species are rather similar with respect to the relative length of chromosomes (Fig. 4). Significant differences occur only in the relative length of the last pair of chromosomes, which form 3.4 % of the total length of the complement for *C. laticeps*, whereas for *G. catostomi* it comprises 5.9 %. The presence of such small chromosomes significantly differing in size from the other elements of the set was observed also in the chromosome set of *Atractolytocestus huronensis* (Jones and Mackiewicz 1969).

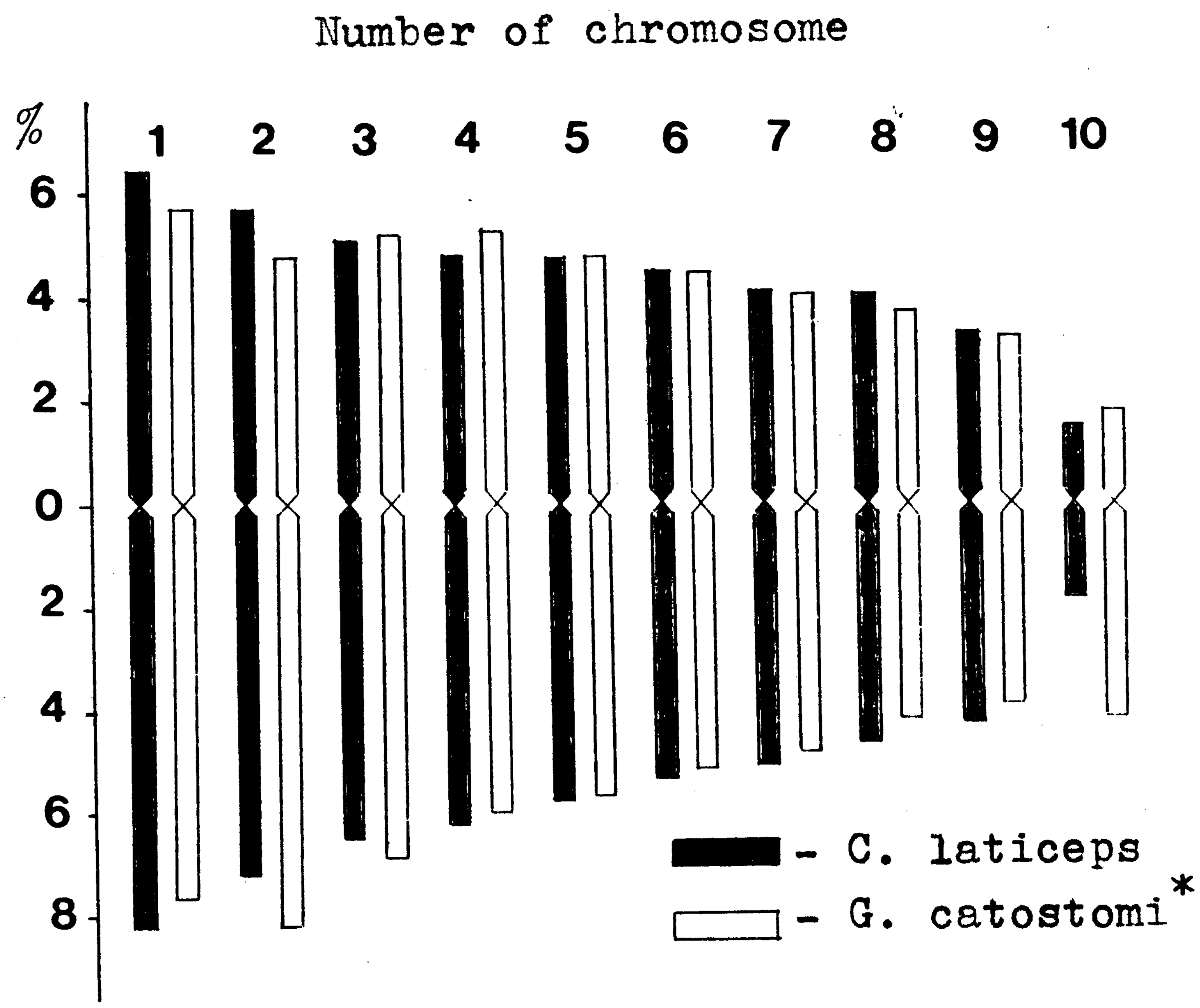

Fig. 4. Idiograms of chromosomes. *Idiogram constructed using the data of Grey and Mackiewicz (1980). 1–10 – chromosome pair number.

It is worth noting that the karyotype of another species of the genus *Glaridacris*, *G. laruei*, consists of 16 chromosomes among which there are 3 pairs metacentric, 1 pair submetacentric, and 4 acrocentric (Grey and Mackiewicz 1974). It differs from *G. catostomi* more significantly than does *C. laticeps*. On the other hand, the structure of the karyotype of *G. laruei* is rather close to that of *Hunterella nodulosa* (Grey and Mackiewicz 1974). These karyological data confirm Mackiewicz's (1982) opinion that a number of genera of caryophyllids, including *Caryophyllaeus* and *Glaridacris*, need reinvestigation.

The rediscovery of the triploid specimen of *C. laticeps* is of interest. Such a specimen with the ability to reproduce parthenogenetically could initiate a triploid population. There is a known triploid species, *Atractolytocestus huronensis* (Jones and Mackiewicz 1969), and triploid population of *G. catostomi* (Grey and Mackiewicz 1980) among caryophyllids, and parthenogenesis is presumed to occur in both cases. The authors of those studies have discussed also the possible mechanisms of triploidisation such as dispermy and formation of gametes with unreduced number of chromosomes. The appearance of an additional chromosome, nonhomologous to others in the triploid karyotype of *C. laticeps*, is difficult to explain. This could be a result of nondisjunction (which is the cause of trisomy) and the ensuring fragmentation of one of the chromosomes in the set.

The existence of two specimens with altered chromosome sets among 23 studied members of the population of *C. laticeps* shows that the lability of genome structure may be characteristic of the species and suggests that further studies may reveal new chromosomal variants.

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